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DATA EVALUATION RECORD[1]

STUDY TYPE: **In vitro Bacterial Gene Mutation** (Bacterial system, *Salmonella typhimurium*)/ mammalian activation gene mutation assay; OPPTS 870.5100 [§84-2]; OECD 471 (formerly OECD 471 & 472).

PC CODE: 016331 **DP BARCODE:** DP410187

TEST MATERIAL (PURITY): Momfluorothrin (95.7% a.i.; Lot No. 9CM0109G)

SYNONYMS: S-1563

CITATION: Kitamoto, S. (2009) Reverse mutation test of S-1563 in bacterial systems. Sumitomo Chemical Co., Ltd., Japan. Report No. RWT-0004, December 1, 2009. MRID 49020026. Unpublished

SPONSOR: Sumitomo Chemical Co., Ltd., Japan

EXECUTIVE SUMMARY:

In duplicate reverse gene mutation assays in bacteria (MRID 49020026), strains of *Salmonella typhimurium* (TA98, TA100, TA1535, and TA1537) and *Escherichia coli* WP2 uvrA were exposed to S-1563 (95.7% a.i.; Lot No. 9CM0109G) in dimethyl sulfoxide (DMSO) at concentrations of 0, 156, 313, 625, 1250, 2500, and 5000 µg/plate in the presence and absence of mammalian metabolic activation using the preincubation procedure. The S9 mix was prepared from the livers of male Sprague-Dawley rats induced with phenobarbital and 5, 6-benzoflavone. The positive control substances, 2-(2-furyl)-3-(5-nitro-2-furyl) acrylamide, sodium azide, 9-aminoacridine and 2-aminoanthracene were used to validate the tester strains and the activity of the S9-mix.

There was no evidence of cytotoxicity up to the highest concentration tested. Precipitation of the test chemical was observed at ≥ 313 µg/plate -S9 and at ≥ 2500 µg/plate + S9. The positive controls induced the appropriate responses in the corresponding strains. S-1563 failed to induce a significant increase in the numbers of observed revertant colonies in the presence or absence of metabolic activation (S9-mix) at any of the levels tested. **There was no evidence of induced mutant colonies over background.**

This study is classified as **Acceptable/Guideline** and satisfies the guideline requirement for Test Guideline OPPTS 870.5100; OECD 471 for in vitro mutagenicity (bacterial reverse gene mutation) data.

1 Disclaimer: The attached Data Evaluation Record is a modified version of the Tier II Summary provided by Sumitomo Chemical Co. Ltd. Portions of this document may have been altered by the EPA reviewer.

COMPLIANCE: A GLP statement was provided.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test material: S-1563
Description: Not stated
Lot/Batch: Lot No. 9CM0109G
Purity: 95.7%
CAS#: 609346-29-4
Stability: Stable for duration of study (analytically determined: 95.6% on August 4, 2009)

The test chemical was stable in dimethyl sulfoxide (DMSO) at 0.015 and 500 mg/mL for 4 hours (at room temperature).

2. Control materials:
Negative: Solvent, DMSO (0.1 mL)
Positive:
Non-activation (-S9): 2-(2-furyl)-3-(5-nitro-2-furyl)acrylamide - TA100, WP2*uvrA* (0.01 µg/plate), TA98 (0.1 µg/plate)
sodium azide – TA1535 (0.5 µg/plate)
9-aminoacridine - TA1537 (80 µg/plate)

Activation (+S9): 2-aminoanthracene – WP2*uvrA* (10 µg/plate), TA1535 and TA1537 (2 µg/plate), TA100 (1 µg/plate), TA98 (0.5 µg/plate)

3. Activation: Commercially-obtained S9-mix (Oriental Yeast Co Ltd, Tokyo)
Animal species: Male Sprague-Dawley rats, 7 week-old, body weight 250±12.1 g
Organ: Liver
Induction: Yes, Phenobarbital (PB) and 5,6-benzoflavone (BF)

S9 Mix composition:

Component	Parts per ml
S9 fraction	0.1 mL
20 mM $MgCl_2$ / 82.5 mM KCl	0.4 mL
Glucose-6-phosphate	5 µmol
NADPH	4 µmol
NADH	4 µmol
200 mM Phosphate buffer (pH 7.4)	0.5 mL

4. Test organism: *Salmonella typhimurium* (TA100, TA98, TA1535, and TA1537) and *Escherichia coli* (WP2*uvrA*). Before use in this study, strains were confirmed to express appropriate phenotypic characteristics (amino acid requirement, UV sensitivity, presence of *rfa* and R factors).

5. Test concentrations: 156, 313, 625, 1250, 2500, 5000 µg/plate for all strains in the presence and absence of S9 mix, in triplicate plates

Note: The preliminary range finding assay investigated the same concentrations; included positive controls; and used triplicate plates with and without S9 activation. Therefore, the range finding assay constitutes a full gene mutation assay.

B. TEST PERFORMANCE

This study was conducted between 14 July 2009 and 27 July, 2009.

1. Test procedure

The assay was performed using the preincubation method with or without S9 mix. Each bacterial frozen stock culture was thawed and cultured for 10 hours. A solution of the test compound (0.1 mL) and bacterial suspension (0.1 mL) was mixed with S9 mix or 100 mM Na-phosphate buffer (0.5 mL), then incubated for 20 minutes at 37ºC with shaking. After addition of 2.0 mL of top agar solution, containing a sterile amino acid solution of 0.5 mM L-histidine and 0.5 mM D-biotin for *S. typhimurium* in 1:10 by volume and a sterile amino acid solution of 0.5 mM L-tryptophan for *E.coli* in 1:10 by volume, the mixture was poured onto a minimal glucose agar plate and incubated at 37ºC for 48 hour. Sterility tests were carried out for the test substance solution and S9-mix and found to be negative.

The number of revertant colonies on each plate was counted using an automatic colony counter (CA-11, System Science Co, Ltd., Tokyo, Japan), and the count values were adjusted to reflect the area. Precipitates of test substance were observed by the unaided eye, and bacterial toxicity was examined under a stereomicroscope. The solvent controls and the positive controls were run concurrently. The range finding assay and the main study were conducted in triplicate.

2. Statistics

A linear regression analysis was used to test for a dose-dependent increase in the number of revertant colonies.

3. Evaluation criteria

A positive response in an experiment would be achieved when a two-fold or greater increase in the mean number of revertant colonies per test plate occurred (over and above that observed for the solvent control plates). A second criterion for a positive result was the observation of a statistically significant dose-related increase in the number of revertant colonies.

II. RESULTS AND DISCUSSION

A. ANALYTICAL DETERMINATIONS

Test material stability was confirmed analytically in the DMSO solvent.

B. CYTOTOXICITY

There was no evidence of cytotoxicity up to the highest dose tested. Precipitates of the test chemical were observed at and above the dose levels of 313 μg/plate without S9 mix and at and above the dose levels of 2500 μg/plate with S9 mix. Results of the sterility tests showed no contamination of the test material solution or S9-mix.

C. MUTATION ASSAY

With all 5 tester strains (TA1535, TA1537, TA98, TA100 and WP2 uvrA) S-1563 failed to induce any significant increase in the numbers of observed revertant colonies in the presence or absence of metabolic activation (S9-mix) at any of the dose levels tested. Representative results from the main assay are summarized in Table 1. The study also showed that all the tester strains were sensitive to their appropriate positive controls.

TABLE 1. Summarized Results of the Main Bacterial Reverse Gene Mutation Assay with S-1563 (Preincubation Method)

Treatment (µg/plate)	S9	Average number of revertants/plate				
		TA1535	TA100	TA1537	TA98	WP2uvrA (pKM101)
DMSO	-	7	86	10	24	18
Positive Control	-	266	636	407	349	115
S-1563						
156	-	8	98	12	19	20
313[a]	-	8	84	7	24	18
625	-	7	95	8	26	18
1250	-	6	90	11	25	23
2500	-	8	86	11	20	19
5000	-	5	96	13	22	19
DMSO	+	8	84	18	25	24
Positive Control	+	172	649	129	195	634
S-1563						
156	+	8	78	20	30	24
313	+	8	98	16	27	33
625	+	9	92	12	24	25
1250	+	7	89	13	30	30
2500[a]	+	7	84	13	23	27
5000	+	9	93	13	21	29

[a] Compound precipitation seen at this and higher levels.
Positive Controls: -S9: 2-(2-furyl)-3-(5-nitro-2-furyl) acrylamide - TA100, WP2*uvrA* (0.01 µg/plate), TA98 (0.1 µg/plate) sodium azide – TA1535 (0.5 µg/plate) 9-aminoacridine - TA1537 (80 µg/plate)
+S9: 2-aminoanthracene – WP2*uvrA* (10 µg/plate), TA1535 and TA1537 (2 µg/plate), TA100 (1 µg/plate), TA98 (0.5 µg/plate)

III. DISCUSSION AND CONCLUSIONS:

A. INVESTIGATORS' CONCLUSIONS:

The study author concluded that S-1563 was not mutagenic in this test system.

B. REVIEWER COMMENTS:

S-1563 was tested up to precipitating concentrations (≥133 µg/plate –S9; ≥2500 µg/plate +S9) but did not induce a mutagenic response in either the presence or the absence of S9-activation in a well-conducted study. Both the nonactivated and the S9-activated positive control induced the expected response in the corresponding bacterial tester strain, thus,

demonstrating the sensitivity of the test system to detect a mutagenic effect. This study is classified as **acceptable /guideline** and satisfies the guideline requirement for the requirement for Test Guideline OPPTS 870.5100; OECD 471 for in vitro mutagenicity (bacterial reverse gene mutation) data.

C. STUDY DEFICIENCIES: None